

LETTERS TO NATURE

anti-fasciclin I or anti-HRP. By contrast, unirradiated embryos injected with dye-labelled antibodies, or embryos injected with unlabelled antibodies, regardless of laser irradiation, showed low defasciculation rates. No effect was seen with laser irradiation of embryos injected with labelled BSA, which presents the dye on a nonspecific protein that does not bind membranes. Similarly, there was no effect when labelled hexaalanine was used; this is a hydrophobic peptide that binds to membranes, but not specifically to proteins, thereby acting as a control for nonspecific damage to the membrane by CALI. Thus, it is insufficient to have the dye present on the cell surface to cause defasciculation; the dye must be linked to a specific protein. CALI-treated embryos also had high defasciculation rates on a per-individual basis. Figure 3b compares the percentage of defasciculated trajectories per animal in laser-treated anti-HRP (open bars) versus laser-treated Malachite green/anti-HRP-injected embryos (filled bars). Malachite green/anti-fasciclin I laser-treated embryos showed a similar distribution to that seen using Malachite green/anti-HRP.

Over 80% of injected embryos survive CALI. The treated limb buds show normal segmentation and growth, with the overall length of the Ti1 axons unaffected (Table 1). The growth cones also appear normal; even defasciculated neurons contact guidepost cells, cross leg segment boundaries and show typical filopodial spread¹². New neurons, such as the femoral chordotonal organ and subgenual organ¹³, are able to differentiate after CALI. CALI does not influence the differentiation of guidepost cells ($n = 202$ limb buds from a single experiment). As CALI causes defasciculation without affecting other phenomena, Ti1 elongation and guidance are not dependent on fasciculation.

Is fasciclin I solely responsible for the axon adhesion observed in these experiments? Elkins *et al.*¹⁴ have shown that *Drosophila* null mutations of fasciclin I have minor effects on the CNS, but that double mutants of fasciclin I and the Abelson tyrosine kinase gene show significant disruption in axonal organization. As CALI can indirectly inactivate a small complex *in vitro* (data not shown), it is possible that a protein closely bound to fasciclin I is also inactivated. Alternatively, regulatory mechanisms may compensate for the loss of fasciclin I in null mutations. This may not occur during the acute inactivation resulting from CALI.

Cell adhesion has been difficult to demonstrate *in situ* during neuronal development because of the paucity of effective inhibiting probes, and the difficulty of controlling their action over a specific time interval. We have employed a novel technique to demonstrate the role of a specific molecule in mediating axon adhesion by converting a binding reagent into an inhibitor. We suggest that CALI could be generally applied in the functional inactivation of other proteins. □

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Interleukin-1 receptor antagonist reduces mortality from endotoxin shock

Kjell Ohlsson*, Peter Björk*, Magnus Bergenfelz*, Robert Hageman† & Robert C. Thompson†

* Department of Surgical Pathophysiology, University of Lund, Malmö General Hospital, S21401, Malmö, Sweden

† Synergen, Inc., 1885 33rd Street, Boulder, Colorado 80301, USA

ABOUT five out of 1,000 patients admitted to hospital develop bacterial sepsis leading to shock¹, the mortality rate for which is high despite antibiotic therapy². The infection results in hypotension and poor tissue perfusion, and eventually leads to the failure of several organ systems. Bacterial endotoxin is thought to be the direct cause of shock in Gram-negative sepsis, because it can cause shock in animals³, and antibodies against endotoxin prevent Gram-negative shock in animals⁴ and in humans⁵⁻⁷. But, the symptoms of septic shock are the result of the actions of host cytokines induced by the endotoxin. The cytokine interleukin-1 has been implicated as an important mediator of septic shock because it can induce tachycardia and hypotension and act synergistically with tumour necrosis factor to cause tissue damage⁸ and death⁹. We now report that a specific interleukin-1 receptor antagonist reduces the lethality of endotoxin-induced shock in rabbits, indicating that interleukin-1 does indeed play an important part in endotoxin shock.

A recombinant human interleukin-1 (IL-1) receptor antagonist (IL-1ra)^{10,11} that blocks the effects of IL-1 *in vitro* has provided a tool for determining the role of IL-1 in animal models of septic shock. The hypotension and leukopenia that follow a single intravenous injection of recombinant human interleukin-1 β (IL-1 β , 15 μ g kg⁻¹) into anaesthetized rabbits⁸ were blocked in a dose-dependent way by the injection of IL-1ra shortly before IL-1 (Fig. 1a, b). The leukocytosis that occurs at later times in conscious rabbits injected with IL-1 β was also blocked (Fig. 1c). These results indicate that IL-1ra should block the effects of endotoxin shock mediated by IL-1 in rabbits.

Because death is a common outcome of septic shock in humans, we tested the effects of IL-1ra in a model of endotoxin shock in rabbits, for which the mortality rate is similarly high. Intravenous injection of *Escherichia coli* endotoxin (0.5 mg kg⁻¹) into rabbits killed eight of ten animals within 48 hours (Fig. 2). Within the first few hours after the injection the animals' fur became ruffled and they became essentially immobile. The animals' breathing became strained and harsh noises could be heard issuing from their lungs. At autopsy, the lungs showed striking pathological changes: On gross examination they were heavy with a liver-like appearance. Light microscopy revealed that the fine alveolar architecture was disrupted, showing oedematous alveolar walls and a massive accumulation of protein and of red and white blood cells (Fig. 3a).

By contrast, nine of ten rabbits receiving a total of 100 mg kg⁻¹ IL-1ra and the endotoxin survived the observation period of 7 days and appeared to make a full recovery (for dosage, see Fig. 2). These animals moved about the cage, and their fur and breathing appeared to be normal, although the animals were less lively than untreated controls. Rabbits of another group treated in the same way were killed after 24 hours so that their lungs could be examined. On gross examination, small areas of surface bleeding but no gross hepatization was seen, and on microscopic examination, there was no evidence of the massive transudation and cellular infiltration seen in the group treated with endotoxin alone (Fig. 3b). The beneficial effects of IL-1ra are dose-dependent. A group of 10 rabbits treated with 30 mg kg⁻¹ IL-1ra had an intermediate level of mortality, and

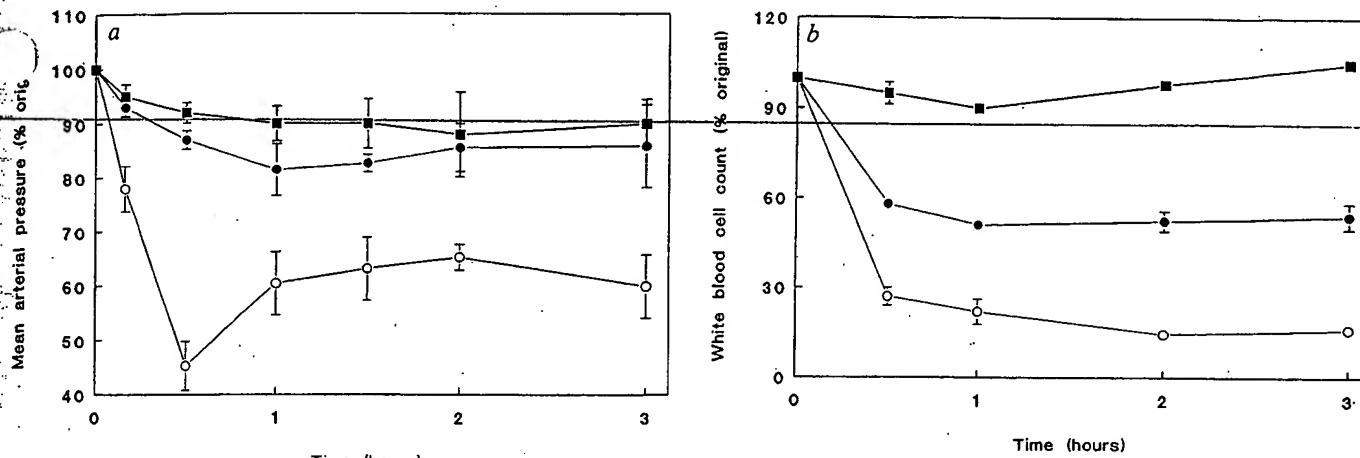


FIG. 1 Effect of IL-1ra on IL-1β-induced changes in mean arterial pressure and white blood cell count in anaesthetized and conscious rabbits, which are as sensitive to endotoxin and IL-1 as humans⁸. Rabbits (blue chinchilla, 2.5 kg; BomMice, Bomholtgaard Breeding and Research Center Ltd, Bomholtvej 10, DK-8680, Ry, Denmark) were anaesthetized with a single injection of 4 mg kg⁻¹ xylazine and 10 mg kg⁻¹ ketamine. Catheters (PE 50) were placed in the left carotid artery and the superior caval vein to allow for the continuous recording of the arterial and central venous pressure. IL-1β and IL-1ra (both prepared from *E. coli* and containing <0.5 U endotoxin per mg protein) were injected into the central venous catheter as a bolus over 1 min. During the observation period, blood was withdrawn from the carotid artery catheter for white blood cell count (originally close to $7 \times 10^9 \text{ L}^{-1}$) and platelet counts, and directly into EDTA for plasma samples. Blood samples removed from the catheters were replaced with the same volume of saline (~5 ml over 3 h). The total volume of saline administered was 1 ml per kg body weight per hour. In one series of IL-1/IL-1ra experiments the animals were allowed to wake up after the catheters were in place. The catheters were secured in the neck to allow repeated measurements of blood pressure and also sampling of blood. Rabbits received either 15 µg kg⁻¹ IL-1β (O), 15 µg kg⁻¹ IL-1β and 1 mg kg⁻¹ IL-1ra (●), or 15 µg kg⁻¹ IL-1β and 4 mg kg⁻¹ IL-1ra (■). Rabbits injected with saline or with 4 mg kg⁻¹ IL-1ra remained haemodynamically stable and showed no significant change in the number of white blood cells throughout the observation period (data not shown).

animals given 10 mg kg⁻¹ IL-1ra had the same level of mortality as untreated animals (Fig. 2).

Large doses of IL-1ra were required to block the action of IL-1 in the IL-1- and the endotoxin-induced diseases, despite the fact that IL-1ra and IL-1 are expected to have similar affinities for the IL-1 receptor on the basis of experiments with

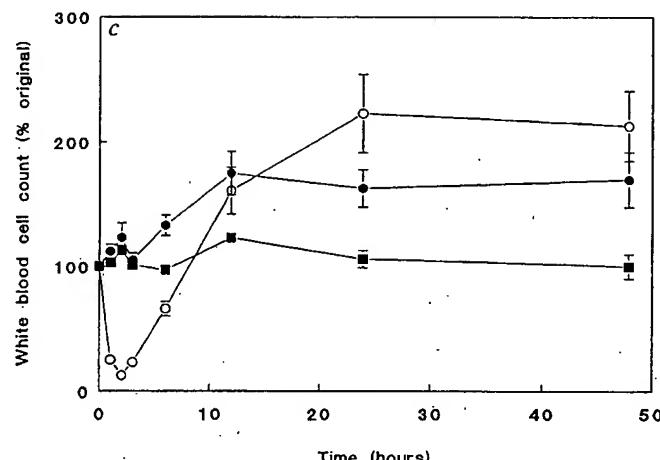
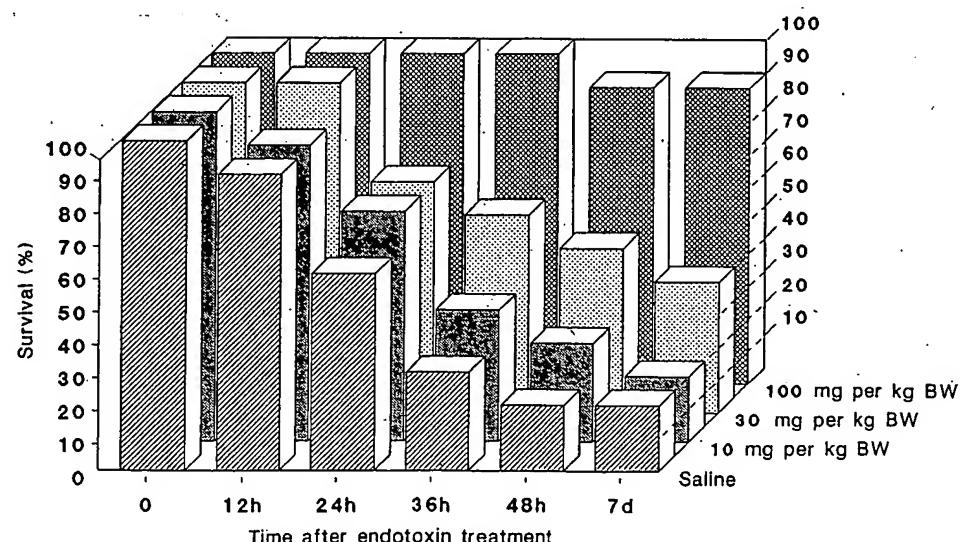


FIG. 2 The effect of IL-1ra on the survival rate in endotoxin-induced shock in rabbits. IL-1ra was injected in equal doses just before the injection of endotoxin (*E. coli* 026, B26; Sigma) and every 2 h thereafter for 24 h. The rabbits were not anaesthetized but were under constant observation for 48 h and were then observed during the daytime for up to 7 days. The animals had free access to water and food. Injections and blood sampling were made through the ear veins. BW, body weight; d, days.

mouse cells¹⁰. In part this high dose indicates that more than 50% of IL-1 binding needs to be blocked for 50% of the biological effects of IL-1 to be blocked. Similar effects occur with cells in culture¹². But the high doses needed are also a consequence of the rapid equilibration and clearance of IL-1ra after intravenous injection. In the group of animals receiving a total of 100 mg



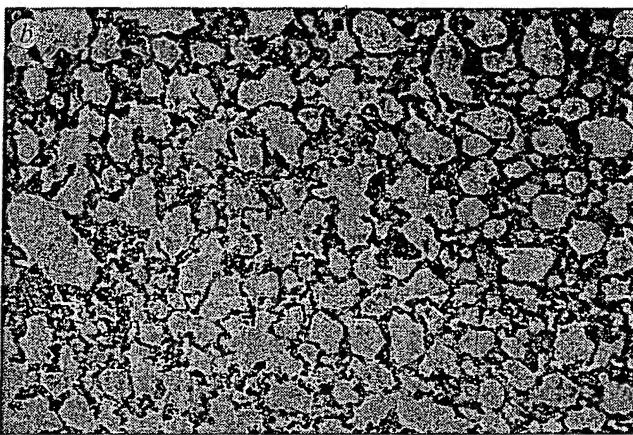
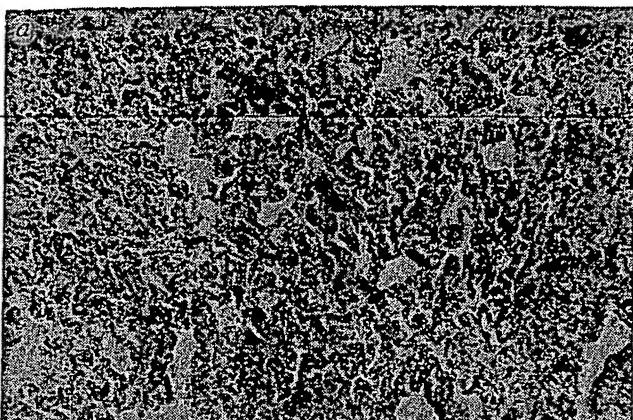


FIG. 3 Microscopic appearance (magnification, 160 \times) of a rabbit lung removed: *a*, immediately after death at 26 h from an animal receiving endotoxin and no IL-1ra; and *b*, 24 h after identical injection of endotoxin from an animal receiving 100 mg rhIL-1ra per kg body weight over the first 24 h. Rabbits were killed with pentobarbital. The tissue specimens were fixed in buffered (pH 7.4) 10% formalin, dehydrated and embedded in paraffin. The tissue samples were analysed with light microscopy with haematoxylin-eosin staining.

IL-1ra per kg body weight, the level of IL-1ra in plasma, as measured by a single radial immunodiffusion assay¹³, varied from between 150 μ g ml⁻¹ 5 minutes after each 7.7-mg kg⁻¹ dose, and 20 μ g ml⁻¹ 2 hours later. These results imply that the quantity of IL-1ra needed to prevent mortality would be considerably reduced if the circulation of the protein could be prolonged.

To determine the time during which IL-1 acts a pathological agent in endotoxin shock we investigated the effects on mortality of delaying the treatment with IL-1ra. When the standard treatment with IL-1ra was delayed for 1 or 2 hours after the endotoxin injection, seven of the eight animals in each group survived the 7-day period whereas three of the four animals in a group not treated with IL-1ra died within 48 hours. These results indicate that IL-1 toxicity can be reversed at least 2 hours after injecting endotoxin. Because IL-1 seems to be a late-acting agent in endotoxin shock, IL-1ra could have therapeutic as well as prophylactic properties in septic shock. This is especially important clinically, where the disease, as measured by hypotension, can be in progress before intervention can be initiated. Experiments are in progress to determine the latest time after administration of endotoxin at which the disease can be prevented.

In light of earlier results implicating tumour necrosis factor as a mediator in endotoxin shock¹⁴⁻¹⁷, the demonstration that IL-1 is also an important mediator shows that the disease probably results from several cytokines acting with additive or syner-

gistic effects. This conclusion is in accordance with IL-1 greatly potentiating the shock action of tumour necrosis factor in mice and rabbits^{8,9}. Other cytokines may also contribute to the pathology of endotoxin shock. But on the basis of the current results we conclude that IL-1 plays an important part in experimental endotoxin shock in animals. It will be worthwhile investigating whether IL-1ra is of practical therapeutic benefit in human septic shock. \square

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Phage antibodies: filamentous phage displaying antibody variable domains

John McCafferty*†, Andrew D. Griffiths*, Greg Winter*‡ & David J. Chiswell†

* MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

† Cambridge Antibody Technology Ltd, Dab Research Laboratories, Babraham Hall, Cambridge, CB2 4AT, UK

NEW ways of making antibodies have recently been demonstrated using gene technology. Immunoglobulin variable (V) genes are amplified from hybridomas or B cells using the polymerase chain reaction, and cloned into expression vectors. Soluble antibody fragments secreted from bacteria are then screened for binding activities (see ref. 1 for review). Screening of V genes would, however, be revolutionized if they could be expressed on the surface of bacteriophage. Phage carrying V genes that encode binding activities could then be selected directly with antigen. Here we show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen and that rare phage (one in a million) can be isolated after affinity chromatography.

The heavy (VH) and light (VL) chain variable (V) domains of the anti-lysozyme antibody D1.3 (ref. 2) associate tightly as an Fv fragment and bind to antigen with a similar affinity to that of the parent antibody³. To allow expression of both domains on the same polypeptide, they were joined by a flexible linker (Gly₄-Ser)₃ (ref. 4), and the single-chain Fv fragment (scFv) cloned into an fd phage vector (fdCAT1) at the N-terminal region of the gene III protein (Fig. 1). The gene III protein is normally expressed at the tip of fd phage (about four copies per virion), is responsible for attachment of phage to the

† To whom correspondence should be addressed.